

AMENDMENTS TO THE CLAIMS

Claim 1. (Currently Amended) A method for the preparation of a strain of evolved ~~micro-organisms~~ *E. coli* for the production of 1,2-propanediol by the metabolism of a simple carbon source, said method comprising:

(a) providing an initial ~~bacterial~~ *E. coli* strain comprising deletion of *tpiA* gene and deletion of at least one gene involved in the conversion of methylglyoxal into lactate;

(b) culturing the initial ~~bacterial~~ *E. coli* strain, under selection pressure in an appropriate growth medium comprising a simple carbon source for a time period sufficient to allow an increase in growth;

(c) causing evolution, in said initial *E. coli* strain, of one or more endogenous genes involved in the biosynthesis pathway from DHAP to methylglyoxal and then to 1,2-propanediol towards evolved genes having an improved 1,2-propanediol synthase activity by applying increasing rates of dilution in such a way as to conserve in the growth medium only those *E. coli* strain that display a growth rate equal to or higher than the imposed rate of dilution to provide an evolved strain; and

(d) selecting and isolating the evolved *E. coli* strain ~~of micro-organisms~~ having an improved 1,2-propanediol synthase activity.

Claim 2. (Previously Presented) The method of claim 1, wherein the gene involved in the conversion of methylglyoxal into lactate is selected from the group consisting of *gloA*, *aldA* and *aldB*.

Claim 3. (Currently Amended) The method of claim 1, wherein the initial *E. coli* strain comprises deletion of genes *gloA*, *aldA*, and *aldB*.

Claim 4. (Currently Amended) The method of claim 1, wherein the initial *E. coli* strain comprises deletion of genes *ldhA*, *pflA*, *pflB*, *adhE* and *edd*.

Claim 5. (Currently Amended) The method of claim 1, wherein the initial *E. coli* strain further comprises a pyruvate dehydrogenase complex.

Claim 6. (Previously Presented) The method of claim 5, wherein the pyruvate dehydrogenase complex has low sensitivity to inhibition by NADH.

Claim 7 – 8. (Cancelled)

Claim 9. (Previously Presented) The method of claim 5, wherein the pyruvate dehydrogenase complex is endogenous.

Claim 10. (Currently Amended) The method of claim 1, wherein one or more heterologous genes *adc*, *ctfAB* ~~*etfA* and *B*~~, and *thl* are introduced into the evolved *E. coli* microorganisms to provide a modified evolved *E. coli* strain.

Claim 11. (Currently Amended) The method of claim 10, wherein the one or more heterologous genes *adc* *ctfAB* ~~*etfA* and *B*~~, and *thl* are from *C. acetobutylicum*.

Claim 12. (Currently Amended) The method of claim 10, wherein the modified evolved *E. coli* strain comprising one or more heterologous genes *adc*, *ctfAB* ~~*etfA* and *B*~~, and *thl* are grown under selection pressure in an appropriate growth medium comprising a simple carbon source in order to cause, in said modified evolved *E. coli* strain, the evolution of one or more genes involved in the conversion of acetyl-CoA and acetate to acetone towards an improved acetone synthase activity, the second generation of resulting evolved microorganisms having an improved 1,2-propanediol synthase activity and an improved acetone synthase activity are then selected and isolated.

Claim 13 – 15. (Cancelled)

Claim 16. (Currently Amended) An evolved *E. coli* strain obtained by the method according to claim 1.

Claim 17. (Currently Amended) The evolved *E. coli* strain according to Claim 35, wherein the evolved *E. coli* strain comprises an *lpd* gene encoding a lipoamide dehydrogenase of the pyruvate dehydrogenase complex, and wherein the *lpd* gene has a point mutation whereby alanine 55 is replaced by valine.

Claims 18 to 21. (Cancelled)

Claim 22. (Currently Amended) An ~~initial~~ *E. coli* bacterial strain of a microorganism comprising a deletion of the gene *tpiA* and a deletion of at least one gene involved in the conversion of methylglyoxal into lactate.

Claim 23. (Previously Presented) The strain of claim 22, wherein the gene involved in the conversion of methylglyoxal into lactate is selected among the group consisting of *gloA*, *aldA* and *aldB*.

Claim 24. (Currently Amended) The strain of claim 22, wherein the initial *E. coli* strain comprises deletion of the genes *gloA*, *aldA*, and *aldB*.

Claim 25. (Currently Amended) The strain of claim 22, wherein the initial *E. coli* strain comprises deletion of the genes *ldhA*, *pflA*, *pflB*, *adhE* and *edd*.

Claim 26. (Currently Amended) The strain of claim 22, wherein the initial *E. coli* strain also contains a pyruvate dehydrogenase complex.

Claim 27. (Previously Presented) The strain of claim 22, wherein the pyruvate dehydrogenase complex has low sensitivity to inhibition by NADH.

Claims 28 – 31. (Cancelled)

Claim 32. (Currently Amended) The evolved *E. coli* strain of claim 16, wherein the at least one gene involved in the conversion of methylglyoxal into lactate is selected from the group consisting of *gloA*, *aldA* and *aldB* to provide a modified evolved *E. coli* strain.

Claim 33. (Currently Amended) The evolved *E. coli* strain of claim 16, comprising deletion of the genes *gloA*, *aldA*, and *aldB* to provide a modified evolved strain.

Claim 34. (Currently Amended) The evolved *E. coli* strain of claim 16, further comprising a modification, the modification comprising deletion of the genes *ldhA*, *pflA*, *pflB*, *adhE* and *edd* to provide a modified evolved strain.

Claim 35. (Currently Amended) The strain of claim 16, wherein the *E. coli* strain comprises a pyruvate dehydrogenase complex.

Claim 36. (Previously Presented) The strain of claim 35, wherein the pyruvate dehydrogenase complex has low sensitivity to inhibition by NADH.

Claim 37 – 38. (Cancelled)

Claim 39. (Previously Presented) The strain of claim 35, wherein the pyruvate dehydrogenase complex is endogenous.

Claim 40. (Currently Amended) The strain of claim 16, comprising one or more heterologous *adc*, *ctfAB* ~~*etfA* and *B*~~, and *thl*.

Claim 41. (Currently Amended) The strain of claim 40, wherein the one or more heterologous gene or genes *adc*, *ctfAB* ~~*etfA* and *B*~~, and *thl* are from *C. acetobutylicum*.

Claim 42 – 45. (Canceled)

Claim 46. (Currently Amended) An evolved *E. coli* strain obtained by the method of Claim 10.

Claim 47. (Currently Amended) The evolved *E. coli* strain according to claim 50, wherein the evolved strain comprises an *lpd* gene encoding a lipoamide dehydrogenase of the pyruvate dehydrogenase complex, and wherein the *lpd* gene has a point mutation whereby alanine 55 is replaced by valine.

Claim 48 - 49. (Canceled)

Claim 50. (Currently Amended) The evolved *E. coli* strain of claim 46, wherein the *E. coli* strain comprises a pyruvate dehydrogenase complex.